Note

Hydroxymethylation of Rutin Induced by Radiolysis as Novel α -Glucosidase Inhibitors

Gyeong Han Jeong and Tae Hoon Kim*

Department of Food Science and Biotechnology, Daegu University, Gyeongsan 38453, Korea.

Received February 28, 2017; accepted April 20, 2017

Rutin, a major flavonoid glycoside found in many higher plants, was exposed at 25kGy of γ -ray and produced three new hydroxymethylated products 2–4 in the C-ring by γ -irradiation. Structures of the new compounds, including absolute configurations, were elucidated based on spectroscopic interpretation (NMR, UV, $[\alpha]_D$, MS, and circular dichroism (CD)). The new unusual rutin derivatives 2 and 3 exhibited significantly enhanced inhibitory effects against α -glucosidase with IC_{50} values of 23.1 ± 1.2 and $11.2 \pm 0.7 \mu M$, respectively, when compared to the parent rutin.

Key words γ -irradiation; rutin; radiolysis; α -glucosidase; hydroxymethylation

Diabetes mellitus, one of the most common chronic diseases worldwide, is a polygenic complex metabolic disease caused by high blood glucose levels. This undesirable disorder constitutes one of the most challenging health problems in recent years and is closely associated with cardiovascular complications.^{1,2)} Serious complications associated with type 2 diabetes mellitus, include peripheral vascular disease, diabetic neuropathy, amputations, renal failure, stroke, and blindness.³⁾ The most effective treatment for type 2 diabetes mellitus is based on achieving optimal blood glucose levels after meals. Recently, delay of dietary monosaccharide absorption *via* inhibition of α -glucosidase activity was shown to be a beneficial strategy for treatment of type 2 diabetes mellitus.⁴⁾ Despite numerous studies on the development of α -glucosidase inhibitors such as acarbose and voglibose, only a few are currently available, and all are sugar mimics with tedious preparation steps as well as serious gastrointestinal side-effects.⁵⁾ Thus, development of effective non-sugar inhibitors of α -glucosidase from natural products has become a current focus of research.^{6,7)}

γ -Irradiation has been demonstrated as an advanced technology applied in food processing and is also known to have major role in the destruction of micro-organisms, because it contains an abundance of reactive species and free radicals such as methoxy ($CH_3O^\cdot$), hydroxy alkyl ($\cdot CH_2OH$), hydrogen ($H^\cdot$), superoxide anion ($O_2^{\cdot-}$), peroxy ($OOH^\cdot$) radicals, and hydroxyl ion ($OH^\cdot$), molecular hydrogen (H_2), and H_2O .⁸⁾ Only a few researches conducted the influence of irradiation on polyphenolic compounds widely distributed in fruits and vegetables. The degradation reaction of ubiquitous flavonoid or formations of other compounds by γ -ray treatment are still uncertain. The change of natural polyphenolic constituents by irradiation is closely related to human health and it is necessary to evaluate their levels under γ -ray exposure conditions because of possible biological effects and safety. Therefore, the changes in the chemical structures and biological efficacies of rutin induced by γ -ray exposure were evaluated.

Rutin is a representative natural flavone glycoside widely distributed in buckwheat, vegetables and fruits and is considered one of the most promising flavone derivatives from a pharmacological point of view.^{9,10)} Previous research has

shown that radiolysis using γ -irradiation is a useful method for bringing about structural modifications and enhancing biological efficacies of natural inactive.^{11–13)} As part of our continuing investigation into creating bioactive compounds from inactive major secondary metabolites, we herein report the radiolytic degradation of rutin under γ -irradiation conditions into newly modified compounds 2–4 exhibiting significantly enhanced inhibitory effects against α -glucosidase compared to the parent rutin.

Results and Discussion

A methanolic sample solution containing rutin was transferred to a container and directly irradiated using cobalt-60- γ -rays, and the transferred products were successively monitored by reversed-phase HPLC analysis. The dried irradiated sample showed significant enhanced inhibitory activity with an IC_{50} value of $37.1 \pm 2.6 \mu g/mL$ against α -glucosidase. Successive column chromatographic purification led to the isolation of three new rutin derivatives, radio-rutinol (2), isoradiorutinol (3), and radiorutinindiol (4) (Fig. 1). The new hydroxymethylated rutin derivatives 2, 3, and 4 were found to contain rare functional groups in the C-ring of rutin.

Compound 2 was obtained as a yellow amorphous powder, $[\alpha]_D^{25} -15.6$ (MeOH). Its molecular formula was determined to be $C_{28}H_{34}O_{17}$ using negative high resolution (HR)-FAB-MS, which showed a pseudomolecular ion peak at m/z 641.1710 $[M-H]^-$. The absorption maxima at 290 nm in the UV spectrum were attributed to a dihydroflavonol nucleus.¹⁴⁾ The sugar composition was determined to be D-glucose and L-rhamnose by microscale acid hydrolysis and directed HPLC analysis of the phenylisothiocyanate derivatives using UV detector based on previously reported convenient method.¹⁵⁾ The presence of the dihydroflavonol skeleton was further suggested by the 1H -NMR spectrum of 2 (Table 1) for diagnostic signals at δ_H 5.45 (1H, s, H-3), an ABX system at δ_H 7.13 (1H, d, $J=1.8$ Hz, H-2'), 6.68 (1H, dd, $J=8.4, 1.8$ Hz, H-6'), and 6.65 (1H, d, $J=8.4$ Hz, H-5'), and two *meta*-coupled AB system at δ_H 6.00 (1H, d, $J=1.8$ Hz, H-6) and 5.80 (1H, d, $J=1.8$ Hz, H-8). The ^{13}C -NMR spectrum of 2 confirmed the presence of a dihydroflavonol skeleton for this molecule, with characteristic C-ring resonances at δ_C 197.1 (C-4), 89.3 (C-2), and 76.4 (C-3).¹⁶⁾ Ad-

* To whom correspondence should be addressed. e-mail: skyey7@daegu.ac.kr

Fig. 1. Structures of New Degraded Products 2–4 of Rutin

Table 1. ^1H - and ^{13}C -NMR Shifts of Compounds 2–4^{a)}

Position	2		3		4	
	δ_{H}^b (J in Hz)	δ_{C} , mult.	δ_{H}^b (J in Hz)	δ_{C} , mult.	δ_{H}^b (J in Hz)	δ_{C} , mult.
2	—	89.3	—	89.3	—	82.3
3	5.45 (s)	76.4	5.45 (s)	78.0	—	143.9
4	—	197.1	—	195.9	—	123.7
5	—	162.2	—	161.5	—	154.5
6	6.00 (d, 1.8)	97.3	6.01 (d, 1.8)	96.6	5.86 (d, 2.4)	98.6
7	—	168.2	—	167.4	—	159.3
8	5.80 (d, 1.8)	96.8	5.81 (d, 1.8)	96.0	5.81 (d, 2.4)	97.1
9	—	163.8	—	162.9	—	155.9
10	—	102.4	—	101.3	—	104.6
11a	3.98 (d, 12.6)	67.4	3.92 (d, 12.6)	66.5	4.17 (d, 12.6)	65.8
11b	3.65 (d, 12.6)	—	3.72 (d, 12.6)	—	4.10 (d, 12.6)	—
12a	—	—	—	—	5.07 (d, 12.0)	56.9
12b	—	—	—	—	4.00 (d, 12.0)	—
1'	—	128.9	—	127.8	—	131.0
2'	7.13 (d, 1.8)	115.5	7.11 (d, 1.8)	114.5	7.00 (d, 2.4)	115.3
3'	—	145.1	—	144.7	—	145.9
4'	—	145.9	—	145.1	—	146.1
5'	6.65 (d, 8.4)	115.8	6.66 (d, 8.4)	115.0	6.71 (d, 8.0)	115.2
6'	6.68 (dd, 8.4, 1.8)	119.7	6.86 (dd, 8.4, 1.8)	118.8	6.80 (dd, 8.0, 2.4)	120.1
1''	4.85 (d, 7.8)	103.8	4.77 (d, 7.8)	105.2	4.59 (d, 7.8)	100.8
2''	3.50 (m)	77.0	3.53 (m)	76.2	3.50 (m)	76.0
3''	3.37 (t, 9.6)	73.5	3.46 (t, 9.6)	74.2	3.44 (t, 9.6)	73.6
4''	3.52 (m)	73.4	3.51 (m)	75.5	3.47 (m)	74.2
5''	3.57 (m)	76.7	3.54 (m)	76.2	3.34 (m)	77.4
6''a	3.97 (dd, 12.0, 2.4)	67.1	4.09 (dd, 12.0, 2.4)	67.1	3.85 (m)	71.7
6''b	3.73 (dd, 12.0, 4.8)	—	3.50 (dd, 12.0, 4.8)	—	3.70 (m)	—
1'''	4.76 (d, 1.8)	101.7	4.73 (d, 1.2)	100.8	4.78 (d, 1.2)	101.1
2'''	3.85 (dd, 3.6, 1.8)	71.4	3.94 (dd, 3.6, 1.8)	70.3	3.88 (m)	71.2
3'''	3.69 (m)	69.2	3.66 (m)	68.5	3.55 (m)	67.6
4'''	3.40 (m)	71.9	3.38 (t, 9.6)	72.4	3.33 (m)	71.2
5'''	3.45 (m)	70.6	3.56 (m)	71.0	3.67 (dd, 9.6, 6.0)	69.3
6'''	1.19 (d, 6.6)	18.1	1.19 (d, 6.0)	17.2	1.25 (d, 6.0)	18.1

a) ^1H -NMR measured at 600 MHz, ^{13}C -NMR measured at 150 MHz; obtained in acetone- d_6 +D $_2$ O with TMS as internal standard. Assignments based on HSQC and HMBC NMR spectra. b) J values (Hz) are given in parentheses.

ditional characteristic signals of two anomeric protons at δ_{H} 4.85 (1H, d, $J=7.8$ Hz, H-1'') and 4.76 (1H, d, $J=1.8$ Hz, H-1'''), as well as 10 oxygen-bearing signals at δ_{H} 3.97–3.37, along with a doublet methyl proton at δ_{H} 1.19 supported the presence of a rutinoside moiety in the molecule. The ^1H -NMR spectroscopic data of **2** also revealed resonances corresponding to a hydroxymethyl substituent at δ_{H} 3.98 (1H, d, $J=12.6$ Hz, H-11a) and 3.65 (1H, d, $J=12.6$ Hz, H-11b). Consistent with these ^1H -NMR observations, the ^{13}C -NMR and heteronuclear single quantum coherence (HSQC) spectra of **2** closely resembled those of the parent rutin,¹⁷⁾ except for the presence

of an oxygenated hydroxymethyl (H-11) and oxymethine (H-3) signals. The linkage point of the hydroxymethyl residue at C-2 in **2** was determined unambiguously from the key heteronuclear multiple bond connectivity (HMBC) spectrum, which showed H-11/C-2, 3, 1' correlations (Fig. 2). The relative stereostructures of the pseudo-axial catechol on C-2 and the pseudo-equatorial sugar moiety on C-3 positions in the C-ring were characterized by the nuclear Overhauser effect spectroscopy (NOESY) spectrum (Fig. 2). The spectrum of **2** displayed correlations between H-3/H-11, 1'', 2', indicating a *trans*-relationship between the phenyl group and the rutinosyl

Fig. 2. Key HMBC and NOESY Correlations of **2**, **3**, and **4**

moiety. While, other possible pseudo-equatorial sugar moiety on C-3 positions can be discarded by the observed NOESY correlation of H-3/H-2'. Furthermore, an energy-minimized model generated by Chem3D Ultra 10.0 based on a presumed configuration matched well with the obtained NOESY correlations (Fig. 2). The circular dichroism (CD) spectrum of **2** showed positive and negative Cotton effects at 291 and 331 nm, respectively, indicating that the absolute configuration of **2** was the 2*R*,3*S*-configuration.¹⁸⁾ Therefore, the absolute structure of **2** was deduced as radorutinol, which is a new radiolysis product as shown in Fig. 1.

The HR-FAB-MS analysis of compound **3** showed a molecular ion peak at m/z 641.1721 $[M-H]^-$, indicating a molecular formula of $C_{28}H_{34}O_{17}$, which is the same as that of **2**. The NMR and UV spectral data of **3** were also nearly identical to those of **2** suggesting that these two compounds are diastereoisomers. The absolute configurations of the component monosaccharides were determined as D-glucose and L-rhamnose by HPLC analysis of their arylthiocyanate derivatives using C_{18} reversed-phase column.¹⁵⁾ The location of the aliphatic residue was unambiguously elucidated by key HMBC correlations of H-11/C-2, 3, 1' correlations (Fig. 2). As shown in Fig. 2, the 2,3-*trans* stereochemistry of the pseudo-equatorial catechol on C-2 and the pseudo-axial sugar moiety on C-3 positions in the aglycone moiety were also tentatively proposed by NOESY correlations of H-3/H-2', 1''. The absolute stereochemistry of 2*S*,3*R* were determined on the basis of negative and positive Cotton effects at 301 and 332 nm, respectively, in the CD spectra based on a comparison with the authentic analogs.¹⁸⁾ Accordingly, the structure of **3** was assigned as isoradorutinol, which is unknown in the literature.

Compound **4** was obtained as a colorless oil, $[\alpha]_D^{20} +21.4$ (MeOH). The HR-FAB-MS of **4** had a molecular ion peak at m/z 655.1879 $[M-H]^-$, which is consistent with the molecular

formula $C_{29}H_{36}O_{17}$. Similarities between the 1D NMR spectra of **4**, including complete analysis of the HSQC, HMBC, and NOESY experiments, and those of **2** and **3** indicated that **4** is also a rutin analogue.¹⁷⁾ Also, absolute configuration of the component monosaccharides were determined after acid hydrolysis as D-glucose and L-rhamnose by convenient sugar analytical method.¹⁵⁾ Comparison of the 1H - and ^{13}C -NMR data of **4** with those of **2** and **3** (Table 1) indicated the presence of an extra hydroxymethyl functionalities at δ_H 5.07 (1H, d, $J=12.0$ Hz, H-12a) and 4.00 (1H, d, $J=12.0$ Hz, H-12b). Further, C-3 and C-4 were found to be unsaturated in **4**, which was implied by the replacement of ^{13}C -NMR signals at δ_C 76.4 (C-3) and 197.1 (C-4) in **2** by signals at δ_C 143.9 (C-3) and 123.7 (C-4) in **4**. These spectral features suggest that **4** is an unusual flavonoid with two hydroxymethyl groups in its dihydropyran ring. The hydroxymethyl groups were located at C-2 and C-4, based on the key HMBC correlations (Fig. 2). Based on the above evidence, the planar structure of new **4** possessing two hydroxymethyl groups modified by γ -irradiation was deduced as radorutindiol, which comprises the unusual 2H-pyran C-ring system.

Among the naturally occurring polyphenols are widely distributed in various food stuffs such as fruits, vegetables, wines, and juices, and divided into several sub classes including flavonoids, phenolic acids, stilbenes, and lignans.¹⁹⁾ Flavonoids are one of the largest plant secondary metabolites and this huge class of natural compounds has been shown to possess a wide spectrum of significant biological benefits, such as antioxidant, anti-inflammatory, antiviral, anticancer, and chemopreventive properties.²⁰⁾ Several naturally occurring flavonoids have been transformed into structurally modified products using microbial and enzymatic methods. A previous study demonstrated that the biotransformed metabolites of rutin derived from *Aspergillus niger* exhibited significantly

Table 2. Inhibitory Effects of Isolated Compounds **2–4** on α -Glucosidase

Compound	IC ₅₀ value (μ M) ^{a)}
1	>500
2	23.1 $\pm$ 1.2
3	11.2 $\pm$ 0.7
4	56.2 $\pm$ 3.5
Acarbose ^{b)}	310.2 $\pm$ 3.6

a) All compounds were examined in triplicate experiments and means within column with different letters differ significantly ($p < 0.05$). b) Used as positive control.

improved antiproliferative effects.²¹⁾

Managing of glucose level in postprandial plasma is essential in the early treatment of diabetes. Enzyme inhibition like α -glucosidase and α -amylase, which are played in the carbohydrate digestion, is a valuable strategy for decreasing postprandial hyperglycemia.²²⁾ According to several *in vivo* studies, α -glucosidase and α -amylase are further verified to be one of the most valuable approaches for diabetes.^{23,24)} In our systemic investigation, structural change of rutin using irradiation processing showed potentially improved α -glucosidase inhibitory activity. As summarized in Table 2, the degraded rutin analogue isoradiorutinol (**3**), having 2*S*,3*R*-stereostructure at the chiral centers, was found to exhibit significantly higher α -glucosidase inhibitory activity with an IC₅₀ value of 11.2 $\pm$ 0.7 μ M than the parent rutin (**1**). Interestingly, another structurally similar radiorutinol (**2**), with 2*R*,3*S*-stereostructure, was found to exhibit relatively weaker inhibitory activity in these bioassays than **3**. Furthermore, the isolated unusual degraded product, radiorutindiol (**4**), showed slightly decreased α -glucosidase inhibitory activity with an IC₅₀ value of 56.2 $\pm$ 3.5 μ M. These findings indicate that the replacement and stereochemistry of the hydroxymethyl group at the C-2 position in dihydroflavonol skeleton may have a positive influence on inhibition of α -glucosidase.

Methanolic radiolysis in the presence of oxygen could produce various free radicals such as methoxy (CH₃O $\cdot$), hydroxymethyl (CH₂OH $\cdot$), hydrogen (H $\cdot$), superoxide anion (O₂ $\cdot^-$), and peroxy (HOO $\cdot$) radicals, leading to formation of oxygen-bearing by-products.²⁵⁾ In this investigation, free radicals induced by the alcoholic radiolysis might have affected donation of hydroxymethyl groups in the molecule. Further systemic investigations are required to verify a hypothesis concerning the reaction mechanism of radiolysis with γ -irradiation of the related compounds.

The current study verifies that rutin is degraded into three new modified products **2**, **3**, and **4**. The structures of the new hydroxymethylated derived from rutin were established based on the spectroscopic data. The new unusual products **2** and **3** containing a hydroxymethyl group at C-2 position exhibited more potent inhibitory activity against α -glucosidase than the parent rutin. Further systematic investigation into the effects of γ -irradiation on biological properties of other natural flavonoids is now in progress.

Experimental

General Experimental Procedures Rutin and acarbose were purchased from Sigma-Aldrich Corp. (St. Louis, MO, U.S.A.). All other chemicals used in this study were of analytical grade. UV spectra were obtained using a Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan) and CD spectra

were run on a JASCO J-720W spectrometer (JASCO, Tokyo, Japan). ¹H- and ¹³C-NMR spectra were measured on a Varian VNS600 instrument (Varian, Palo Alto, CA, U.S.A.) operated at 600 and 150 MHz, respectively. Chemical shifts are given in δ (ppm) values relative to those of the solvent acetone-*d*₆ (δ _H 2.04, δ _C 29.8) on a tetramethylsilane (TMS) scale. The standard pulse sequences programmed into the instruments were used for each 2D measurement. The J_{CH} value was set at 8 Hz in the HMBC spectra. FAB-MS were obtained on a Micro Mass Auto Spec OA-TOF spectrometer (Micromass, Manchester, U.K.). Column chromatography was performed using Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan) and YMC GEL ODS AQ 120-50S (YMC Co., Kyoto, Japan). TLC was performed on Kieselgel 60 F₂₅₄ plates (0.25 mm layer thickness, Merck, Darmstadt, Germany), and spots were detected by UV irradiation (254, 365 nm) as well as spraying with 10% H₂SO₄ reagent.

Sample Preparation and Isolation Procedure A cobalt-60- γ -source with an activity of approximately 215 kCi with a dose rate of 25 kGy/h was used for irradiation. A sample solution of rutin (1.0 g) in MeOH (1.0 L) in capped vials was irradiated with γ -ray 25 kGy (absorbed dose). The irradiated rutin were directly dried and successively monitored by reversed-phase HPLC analysis and exhibited most improved inhibitory activity with an IC₅₀ value of 37.1 $\pm$ 2.6 μ g/mL against α -glucosidase inhibition assay using a previously reported method.²⁶⁾ A part of the irradiated sample (700 mg) was subjected to column chromatography over a Toyopearl HW-40 column (coarse grade; 2.8 cm i.d. $\times$ 32 cm) and a YMC GEL ODS AQ 120-50S column (1.5 cm i.d. $\times$ 36 cm) with H₂O containing increasing amounts of MeOH in a stepwise gradient to yield pure new compounds **2** (9.5 mg, t_R 10.3 min), **3** (9.8 mg, t_R 11.6 min), and **4** (1.7 mg, t_R 7.8 min) (Fig. 1). HPLC analysis was carried out on a YMC-Pack ODS A-302 column (4.6 mm i.d. $\times$ 150 mm; YMC Co., Ltd.) and the solvent system consisted of a linear gradient that was initiated with MeCN in 0.1% HCOOH–H₂O (detection: UV 280 nm; flow rate: 1.0 mL/min; at 40°C), increased to 20% MeCN over 15 min, and then increased to 100% MeCN over 20 min.

Radiorutinol (**2**)

Yellow amorphous powder. [α]_D²⁵ –15.6 (c =0.1, MeOH); UV (MeOH) λ_{max} (log ϵ): 220 (2.02), 290 (1.58) nm. CD (MeOH) λ_{max} ($\Delta\epsilon$): 291 (+9.71), 331 (–7.43) nm. ¹H- and ¹³C-NMR, see Table 1. FAB-MS m/z 641 [M–H] $^-$, HR-FAB-MS m/z 641.1710 [M–H] $^-$ (Calcd for C₂₈H₃₃O₁₇: 641.1718).

Isoradiorutinol (**3**)

Yellow amorphous powder. [α]_D²⁵ –33.7 (c =0.1, MeOH); UV (MeOH) λ_{max} (log ϵ): 220 (2.03), 290 (1.57) nm. CD (MeOH) λ_{max} ($\Delta\epsilon$): 301 (–7.43), 332 (+7.51) nm. ¹H- and ¹³C-NMR, see Table 1. FAB-MS m/z 642 [M] $^-$, HR-FAB-MS m/z 642.1793 [M] $^-$ (Calcd for C₂₈H₃₄O₁₇: 642.1796).

Radiorutindiol (**4**)

Yellow amorphous powder. [α]_D²⁵ +21.4 (c =0.1, MeOH). UV (MeOH) λ_{max} (log ϵ): 215 (3.42), 280 (1.65) nm. ¹H- and ¹³C-NMR, see Table 1. FAB-MS m/z 655 [M–H] $^-$, HR-FAB-MS m/z 655.1879 [M–H] $^-$ (Calcd for C₂₉H₃₅O₁₇: 655.1874).

Determination of Sugar Configuration Compounds **2–4** (0.5 mg each) were heated in 0.5 N HCl (0.1 mL) at 90°C for 2 h and the solution was neutralized. After drying *in vacuo*, the residue was dissolved in pyridine (0.1 mL), L-cysteine methyl ester hydrochloride (0.5 mg) was added, and the mixture was

warmed at 60°C for 1 h. A 0.1 mL solution of *o*-torylisothiocyanate (0.5 mg) in pyridine was added to the reaction mixture, which was warmed at 60°C for 1 h. The mixture was directly analyzed by reversed-phase HPLC. HPLC analysis was carried out on a Cosmosil 5C₁₈-MS-II column (4.6 mm i.d.×250 mm; Nacalai Tesque Inc., Japan) with isocratic mode of 25% MeCN in 50 mM H₃PO₄ (detection: UV 250 nm; flow rate: 0.8 mL/min; at 40°C). Under above analytical conditions, authentic D/L-glucose, D/L-rhamnose gave HPLC peaks at *t_R* 17.8/16.4 and 15.7/30.2 min, respectively. The retention times of the reacted sugars obtained by acid hydrolysis gave similar results as those of authentic sugar derivatives.

Inhibitory Effects of α -Glucosidase A previously reported method²⁶⁾ with minor modifications was used to evaluate α -glucosidase inhibitory activities of the compounds. Briefly, the reaction mixture consisted of enzyme solution (0.02 unit of α -glucosidase, 50 μ L), substrate (1 mM *p*-nitrophenyl- α -D-glucopyranoside, 50 μ L) in 50 mM phosphate buffer (pH 7.0), and test sample in 5% dimethyl sulfoxide (DMSO) (10 μ L). After incubation at 37.5°C for 20 min, 2 M NaOH was added to stop the reaction. The amount of released *p*-nitrophenol was measured at 410 nm using a UV microplate reader (Infinite F200; Tecan Austria GmbH, Grödig, Austria). The IC₅₀ value was calculated by linear regression analysis of inhibitory activities under the assay conditions. Acarbose was used as a positive control, and all assays were carried out in triplicate.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

References

- 1) Anselmino M., *J. Diabetes Complications*, **23**, 427–433 (2009).
- 2) Fava S., Fava S., *Expert Rev. Cardiovasc. Ther.*, **6**, 859–872 (2008).
- 3) Israili Z. H., *Am. J. Ther.*, **18**, 117–152 (2011).
- 4) Scully T., *Nature* (London), **485**, S2–S3 (2012).
- 5) Asano N., Tomioka E., Kizu H., Matsui K., *Carbohydr. Res.*, **253**, 235–245 (1994).
- 6) Benalla W., Bellahcen S., Bnouham M., *Curr. Diabetes Rev.*, **6**, 247–254 (2010).
- 7) Ryu H. W., Cho J. K., Curtis-Long M. J., Yuk H. J., Kim Y. S., Jung S., Kim Y. S., Lee B. W., Park K. H., *Phytochemistry*, **72**, 2148–2154 (2011).
- 8) Bilal Butt S., Qureshi R. N., *Radiat. Phys. Chem.*, **77**, 768–774 (2008).
- 9) Pashikanti S., de Alba D. R., Boissonneault G. A., Cervantes-Laurean D., *Free Radic. Biol. Med.*, **48**, 656–663 (2010).
- 10) Koda T., Kuroda Y., Imai H., *Food Chem.*, **28**, 629–634 (2008).
- 11) Park C. H., Chung B. Y., Lee S. S., Bai H. W., Cho J. Y., Jo C., Kim T. H., *Bioorg. Med. Chem. Lett.*, **23**, 1099–1103 (2013).
- 12) Kim T. H., Kim J. K., Ito H., Jo C., *Bioorg. Med. Chem. Lett.*, **21**, 1512–1514 (2011).
- 13) Marfak A., Trouillas P., Allais D. P., Calliste C. A., Cook-Moreau J., Duroux J. L., *Radiat. Res.*, **160**, 355–365 (2003).
- 14) Wu T. S., Hsu M. Y., Kuo P. C., Sreenivasulu B., Damu A. G., Su C. R., Li C. Y., Chang H. C., *J. Nat. Prod.*, **66**, 1207–1211 (2003).
- 15) Tanaka T., Nakashima T., Ueda T., Tomii K., Kouno I., *Chem. Pharm. Bull.*, **55**, 899–901 (2007).
- 16) Shen C.-C., Chang Y.-S., Hott L.-K., *Phytochemistry*, **34**, 843–845 (1993).
- 17) Wang M., Kikuzaki H., Csiszar K., Boyd C. D., Maunakea A., Fong S. F., Ghai G., Rosen R. T., Nakatani N., Ho C. T., *J. Agric. Food Chem.*, **47**, 4880–4882 (1999).
- 18) Nonaka G., Goto Y., Kinjo J., Nohara T., Nishioka I., *Chem. Pharm. Bull.*, **35**, 1105–1108 (1987).
- 19) Wang S. Y., Camp M. J., Ehlenfeldt M. K., *Food Chem.*, **132**, 1759–1768 (2012).
- 20) Harborne J. B., Williams C. A., *Phytochemistry*, **55**, 481–504 (2000).
- 21) You H. J., Ahn H. J., Ji G. E., *J. Agric. Food Chem.*, **58**, 10886–10892 (2010).
- 22) Kim Y. M., Jeong Y. K., Wang M. H., Lee W. Y., Rhee H. I., *Nutrition*, **21**, 751–761 (2005).
- 23) Etxeberria U., de la Garza A. L., Campión J., Martinez J. A., Milagro F. I., *Expert Opin. Ther. Targets*, **16**, 269–297 (2012).
- 24) van der Laar F. A., *Vasc. Health Risk Manag.*, **4**, 1189–1195 (2008).
- 25) Getoff N., Ritter A., Schwörer F., Bayer P., *Radiat. Phys. Chem.*, **41**, 797–801 (1993).
- 26) Kim Y. M., Wang M. H., Rhee H. I., *Carbohydr. Res.*, **339**, 715–717 (2004).